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FROM
THE JOURNAL OF EXPERIMENTAL MEDICINE
VOL. III, No. 1, 1898

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PLATE IX.

Since the discovery of the hæmocytozoa of malarial fever considerable attention has been paid to parasites of a similar nature which have been found in various animals. Those which inhabit the red corpuscles of the blood of birds have excited particular interest because of their close resemblance to the malarial organisms. While numerous European observers, for the most part clinicians and pathologists rather than zoologists, have studied the parasites occurring in the blood of birds, no investigations have up to the present time been made in this country.* Dr. Thayer suggested to Mr. MacCallum and the author to make a study of such organisms as were to be found in the blood of birds captured in the neighborhood of Baltimore. We devoted a portion of the summer of 1896 to this work, which has been done in the Clinical Laboratory of the Johns Hopkins University and Hospital. To Dr. Thayer we are indebted for the many suggestions which he has given us.

History.—Almost ten years before the discovery of the malarial organism by Laveran in 1880, Ray Lankester † had observed in the blood of frogs very small worm-like bodies free in the plasma. Butschli,‡ in 1876, mentions the fact that he has frequently found in the red corpuscles of frogs, peculiar bodies occurring at the side of the nucleus; his figures show that these bodies represent an intracorpuscular stage in the life history of the organism which Lankester saw in the free state. This is apparently the first observation of a parasite within the red corpuscles —a hæmocytozoon.

* A brief preliminary report of our observations appeared in the proceedings of the Johns Hopkins Hospital Medical Society, *Bull. of the Johns Hopkins Hospital*, March, 1897, No. 72, p. 52.

† *Quarterly Jour. of the Microsc. Sciences*, xi (1871).

‡ *Abhandl. der Senkenberg. naturf. Gesellsch.*, 1876, p. 261.

Ignorant of these observations, Gaule * later described in the red corpuscles of the frog bodies which he speaks of as "Würmchen." These he believed to be formed from the protoplasm of the corpuscle as a result of degenerative changes; he gave them the name *Cytozoa*. Soon afterwards, however, Ray Lankester † established the parasitic nature of these structures and their identity with the organisms which he had previously observed.

Danilewsky ‡ (2) in 1885 described, for the first time, intracorpuscular parasites in the blood of lizards, tortoises and birds. The organisms which he has found in the bird differ markedly from those in cold-blooded animals—frogs, lizards, tortoises. Most conspicuous at first sight is the fact that the latter do not transform the hæmoglobin into pigment granules; other points of difference are, however, not wanting. We have to do at present only with the organisms found in the blood of birds, these most closely resembling the malarial parasites of human beings.

In the numerous articles which Danilewsky has written a variety of forms have been described:

As *pseudo-vacuoles* he describes bodies which appear as clear areas of variable size and shape within the red corpuscle; in the pseudo-vacuoles he finds black granules of pigment, a product of the transformation of the hæmoglobin.

As *pseudo-vermiculi* he describes elongated bodies with an active wriggling motion which are found free in the plasma. He observed two forms: one non-pigmented similar to the organisms found in cold-blooded animals; the other arising from pigmented pseudo-vacuoles which have escaped from red corpuscles.

To the pigmented flagellate organism, which he describes as a distinct species of parasite, he gives the name *Polymitus avium*. These develop as pseudo-vacuoles in the red corpuscles and subsequently undergo the process of flagellation.

At times the flagella become detached and maintain their active motion for a time; to these free flagella he has applied the term *pseudo-spirilla*.

Although Danilewsky had, for a long time, no opportunity of examining malarial blood, he was much impressed with the similarity between the organisms which he had observed and those which had been described by Laveran, and Marchiafava and Celli. The full-grown parasites,

* *Arch. f. Anat. u. Phys.*, Phys. Abth., 1880, pp. 57, 375; ibid., 1881, p. 297.

† *Quarterly Jour. of the Microsc. Sciences*, Jan. 1882, p. 53.

‡ References to literature at end of this article.

pseudo-vacuoles, in the birds examined by him were approximately half-moon-shaped bodies which suggested the crescents of irregular malarial fever; the flagellate forms differed but little from those found in man. In a short article, published in 1886 (4), he raises the question whether or not these organisms are identical, and answers it in the affirmative.

Grassi and Feletti (12) found, particularly in sparrows (*Passer hispaniolensis*) and in pigeons, what they regarded as two distinct types of organism. One form was the half-moon-shaped parasite already described by Danilewsky; this they believed to be closely related to the crescents of malarial fever. They established the genus *Laverania* to include the two forms to which they gave the specific names, *Laverania danilewskyi* (of the bird), *Laverania malariae* (of man).

The second type of parasite, described for the first time by them, was a smaller organism of irregular shape occupying one end of the corpuscle. This body they regarded as closely related to the amœboid forms of the malarial parasites; they established, therefore, the genus *Haemamoeba* to include them. They find in birds two common species of these small, irregular parasites, which they have termed the *Haemamoeba subpraecox* and *Haemamoeba relicta*. They have further described a third type of organism which they observed in a single bird; to this they gave the name *Haemamoeba immaculata*, owing to its freedom from pigment granules.

Celli and Sanfelice (1) have also attempted to bring the parasites found in the blood of birds into conformity with those of the malarial fevers. They base their classification of the parasites which they have observed upon the rapidity with which these organisms undergo their cycle of development. They find:

(1) Parasites with slow development, reproducing after eight days of growth. Morphologically these are the half-moon-shaped bodies of Danilewsky. Celli and Sanfelice think that they correspond to the quartan organism of man.

(2) Parasites with a shorter period of development.

(3) Parasites with rapid development.

Of the two latter varieties they could not determine the exact length of the cycle of development. Morphologically they are irregular bodies lying at one end of the corpuscle. They differ from one another, according to Celli and Sanfelice, in the size of the sporulating form, which in those with quick development is smaller and divides into fewer segments.

The second variety of parasite, that with a cycle of development of mean duration, they think, corresponds to the tertian organism; the third variety, that with rapid development, to the organism of æstivo-autumnal

fever. Although Celli and Sanfelice insist upon an analogy between these forms and those of man, they do not assert that they are identical.

They found one or more of these varieties of organism in various species of birds; two varieties may co-exist within the same host. In some instances, where they found for a number of days but one variety, there appeared later a second, which became eventually far more abundant and finally replaced the first. They are in fact inclined to believe that one form can change into another; that they are dealing with a single polymorphous parasite. They have never succeeded in transferring infection from one bird to another of a different species. Hence they come to the conclusion that despite the great morphological similarity between the parasites of different birds they are yet separate organisms, each species of bird having its own variety of parasite which will flourish in this species alone.

Danilewsky (7) has attempted to demonstrate the existence in infected birds of symptoms analogous to those of the malarial fevers. He describes an acute and a chronic "malarial" infection in birds. The acute condition is characterized by rise of temperature, loss of weight and general symptoms of illness. In the chronic infection there is no rise of temperature; after death, however, the spleen is found to be much enlarged and pigmented. The acute infection is caused, he thinks, by the small irregularly-shaped parasite described by Grassi and Feletti; the chronic by the half-moon-shaped bodies. In his later articles Danilewsky does not speak with definiteness of the identity of the birds' parasites with the malarial organisms of human beings; he maintains, however, that the varieties described are very similar if not identical.

Labbé (20), more recently, has carefully studied the intracorpuscular parasites of the blood of vertebrates from a purely zoological point of view. He classifies the various organisms that have been described by himself and previous observers according to structural peculiarities. In birds' blood he recognizes the two types distinguished by Grassi and Feletti. The names which he has assigned to them do not imply any necessary correspondence with the malarial parasites of man. To the small irregular forms he gives the generic name, *Proteosoma*. Of this genus he finds but a single species, *Proteosoma grasii*. To the elongated forms, which lie curved about one side of the nucleus of the corpuscle, he gives the name *Halteridium*. Of these also he finds but a single well-marked species, *Halteridium danilewskyi*.

A large number of inoculation experiments have been performed. Celli and Sanfelice, Laveran and Labbé claim to have successfully inoculated organisms from the blood of one bird into another of the same

species. Grassi and Feletti were never successful in such attempts. Di Mattei (11) has more recently performed a large number of inoculation experiments with pigeons. He injected by various methods blood of infected birds into those not infected. In 83 experiments he obtained universally negative results. He injected blood of malarial patients into 16 pigeons with no consequent infection. The same result was obtained in four instances in which he injected the blood of infected birds into the arm veins of healthy men. He concludes, therefore, that, though the parasites of birds morphologically resemble the organisms of malaria, they are essentially different.

PERSONAL EXPERIENCES.

Our studies were carried on in the months of June, July, August and September, 1896. One hundred and twenty-five birds were examined. Blood was drawn from the wing, and both fresh and dried and stained specimens were studied.

About one hundred birds were examined which had been captured in the neighborhood of Baltimore. Of these the greater number, eighty, were English sparrows (*Passer domesticus*); of the remainder, twelve were red-wing blackbirds (*Agelaius phoeniceus*). The others belonged to a variety of species. The great majority of these birds were obtained from places outside the city and near the Patapsco River or Chesapeake Bay, districts notoriously malarious. Of the eighty English sparrows twenty-one were caught in the city on the Hospital lawn; none of these showed infection. Of the remaining fifty-nine sparrows one in twelve was infected. These figures are almost too small to offer even a suggestion as to the influence of locality on the frequency of infection.

Among the red-wing blackbirds infection seems much more common; of twelve birds, six were infected.

In fifteen birds altogether the blood showed intracorpuscular parasites in varying abundance. Among these birds the following species were represented: *Passer domesticus, Agelaius phoeniceus, Melospiza georgiana, Melospiza fasciata.* It is evident that the observations were not made upon a sufficiently large scale to arrive at any very general conclusions as to the species prone to infection, or as to the local conditions favoring infection. Nevertheless, they indicate, prob-

ably with sufficient accuracy, the commonest varieties of parasites found in this locality.

While at his home in Dunnville, Ontario, not far from Toronto, Mr. MacCallum examined a number of birds. Of twenty-five, in five were found intracorpuscular parasites. At present there is little if any malarial fever in this locality, although at one time the disease was more common. Of two great-horned owls (*Bubo virginianus*) examined one was found to have a most severe infection. In four of five crows (*Corvus americanus*) the blood contained similar organisms. The birds whose blood was negative included a few individuals of a variety of species.

Types of Organism. Two varieties of organism may be distinguished among the intracorpuscular parasites which we have studied. These correspond to the two forms described by Grassi and Feletti and subsequent observers. Characteristic of each group is the morphology of the full-grown organism. In the one an irregularly shaped body, containing pigment granules, occupies one end of the red corpuscle, while the nucleus of the corpuscle is displaced from its normal central position into the opposite end. The other group is characterized by the fact that the full-grown parasite is an elongated pigmented body lying along one side of the normally situated nucleus and curving more or less over its two extremities; this is the so-called halter-shaped form—*Halteridium* of Labbé. Both develop from very small, non-pigmented, approximately spherical bodies.

In the first variety as the body grows it increases in size gradually in all dimensions, and in growing the parasite exerts sufficient force to cause the displacement of the nucleus. In the second, whether as a result wholly due to peculiarities of the organism or to forces acting within the corpuscle, such, for example, as resistance to the displacing of the nucleus, growth takes place more rapidly along that axis which corresponds with the long diameter of the corpuscle. We may distinguish, then, according to their method of growth, two forms, one irregular and the other elongate. This is their most conspicuous difference; other peculiarities of the two forms are, however, not lacking.

In the majority of infected birds either one or other form was

observed alone. Nevertheless, in three birds the two varieties were present at the same time. Hence the possibility suggests itself that the two forms may represent different phases in the life history of the one organism. This view, as we have mentioned, has been supported by Celli and Sanfelice. In birds where one or the other form is present it is, however, possible to trace the whole cycle of development from hyaline non-pigmented bodies to full-grown organisms; it cannot be doubted that the full-grown parasites of both forms segment to form new hyaline bodies. The cycle of development is very different in the two forms and leaves little ground for the belief that one form may appear as a stage in the development of the other. Moreover, in the human being where the multiplicity of species of the intracorpuscular parasites, though not absolutely undisputed, still rests upon much stronger evidence than does that of the organisms of birds, the possibility of an infection with two kinds of malarial parasites at the same time is well known. The frequency of such infection in this locality, for example, is shown by the fact that out of 542 cases of malarial fever studied by Thayer and Hewetson,* there were eleven instances of double infection. The three instances above mentioned, in which the two forms were simultaneously present in the same blood, occurred in blackbirds. Of the blackbirds which we examined one-half contained intracorpuscular parasites. It is not surprising, then, that, where infection is so common, combined infections should be more frequent. Among the English sparrows, of which only one in fifteen was infected, double infection was not observed. For these reasons, then, we believe the two forms to be distinct, and shall therefore consider them separately.

Irregular Parasite (*Proteosoma of Labbé*). The earliest phase of development of this organism is represented by an approximately round, clear body situated within the red corpuscle. Since the body is refractive and gives no indication of amœboid movement, it is hardly to be distinguished in the fresh blood from the vacuoles, which are not infrequently found in the red corpuscles. Nevertheless, in specimens of blood, dried, hardened and stained with eosin and methy-

* *The Johns Hopkins Hospital Reports*, Vol. V.

lene blue according to the method of Romanowsky, modified somewhat by Heppener,* the earliest stage of the parasite is clearly recognizable as a very small body of an oval, round or slightly irregular shape (Plate IX, Fig. 1). The way in which these structures stain is fairly constant; the peripheral portion takes a rather deep blue color, while within this blue rim the substance of the parasite is almost completely unstained.

As the parasite grows larger it acquires pigment, at first a fine black granule situated within the external staining portion of the organism (Plate IX, Figs. 2-5). As the parasite grows, other granules appear. These are, in the great majority of cases, collected into a more or less loose clump, situated as a rule near the periphery of the body; at times, indeed, one sees a particle of pigment lying apparently upon the surface of the parasite. In the neighborhood of the main clump there are usually one or more isolated granules. In the large forms these may sometimes upon careful observation be seen to have a slow motion away from or towards the main mass; they may merge into it, while the clump itself may slowly change its form, presumably by a rearrangement of the constitutent particles. This collection of the pigment into a single clump in the half- and full-grown forms is almost characteristic of the irregularly shaped organisms; at times, however, one sees parasites with discretely scattered pigment granules. The shape of these pigmented bodies is variable; short blunt processes are very frequent. Although we have carefully watched these organisms in fresh blood for a long time, both at room temperature and on the warm stage at 40-41° C., we have not been able to convince ourselves of any change in their shape. Nevertheless, the irregularity in the shape of the bodies and the frequency with which processes are seen, make it probable that a very slow amœboid movement does occur.

In stained specimens the distinction between the peripheral deeply stained rim and the central unstained portion is lost in these larger forms; the whole body takes a fairly deep color. The stain is, however, not uniform, the body having a somewhat granular appearance; we have not seen the definite granules of staining material which can

* *Med. Pribav. K. Morsk. Sborn.*, Jan. and Feb., i (1895), 67.

be made out in certain varieties of the elongate organism (p. 92). Often one may distinguish an approximately round, sharply marked off area, which is almost unstained at times. This may contain one or more faintly staining bodies. This structure, possibly always present in the larger forms, though obscured in some instances by a too deep stain, is what has been described as a vesicular nucleus containing nucleoli.

The growing organism, which at first may be situated in any part of the corpuscle, comes to occupy one or the other extremity. Not infrequently two parasites, or it may be more, are present in the same corpuscle. In such cases they are almost always of the same size; occasionally, however, one is much larger than the other. As the parasite increases in size a change takes place in the position of the nucleus of the corpuscle; it is deflected from its normal longitudinal and central position until it not infrequently comes to lie almost or quite at right angles to the long axis of the corpuscle and at the same time nearer one extremity.

One would suppose that the displacement of the nucleus was the mechanical effect of the growth of the parasite, and, in fact, in many cases the parasite fills the whole of one extremity of the corpuscle with one of its sides flattened out against the nucleus which lies crowded transversely into the other extremity. Not infrequently, however, as Labbé has pointed out, a small parasite may be at one end of the corpuscle, while the nucleus is displaced into a transverse position, although it is touched at no point by the parasite (Plate IX, Fig. 2). The explanation of the process in such cases does not seem, then, to lie altogether in a mechanical displacement of the nucleus, but apparently involves a consideration of the internal structure of the red corpuscle and of the forces that hold the nucleus in place. Upon these points, beyond the obvious fact that there is a marked disturbance of such forces, we can say nothing.

It is interesting in this connection, that one not infrequently may come across parasites surrounded by a rim of red blood corpuscle from which the nucleus is absent, having been apparently extruded (Plate IX, Figs. 6 and 7); this picture we have observed only with the variety

of organism now under consideration. In such cases the remains of the corpuscle are somewhat distorted; often one extremity is drawn out into a point. The stroma of the corpuscles containing the half- and full-grown forms is not decolorized, nor is any alteration of the nucleus to be seen.

With the irregular organism the whole process of segmentation, which is closely similar to that of the malarial parasites of man, takes place in the circulating blood. Bodies representing the first stages of the process are at times met with in great abundance. The parasite assumes an approximately round form; the pigment collects into a solid block which occupies the centre of the body. The outline of the parasite is not perfectly even, but shows more markedly, in proportion as the process has advanced, indentations at regular intervals, which give the whole body somewhat the appearance of a rosette. Situated near the periphery is a circle of minute refractive dots, each of which occupies a position midway between two of the peripheral indentations. The indentations at the periphery become more marked and the organism divides into a variable number, roughly speaking five to twenty small bodies, which assume a circular outline. The size and shape of the segmenting bodies vary. In the larger forms within the peripheral row an internal row of segments about the pigment clump may be found.

Very frequently the corpuscle containing these large bodies shows certain changes; there is some decolorization of the stroma; the corpuscle becomes less oval, the short diameter being increased at the expense of the long; the nucleus of the corpuscle also is often apparently swollen, becoming round and less elongated. Finally, the newly formed bodies become free, and in fresh specimens of blood solid pigment clumps surrounded by groups of small spherical bodies may be seen free in the plasma. Stained by the method of Romanowsky, the segmenting bodies always take a deep color. When the process has advanced almost to division into separate segments, the body may be seen to consist of a number of approximately round, clear areas surrounded by deeply stained protoplasm; within the clear spaces may be seen blue specks which represent possibly the refractive dots of the

fresh body (Plate IX, Fig. 9). After complete segmentation there result a number of round bodies staining at the periphery and clear in the centre, identical in appearance with the youngest intracorpuscular parasites (Plate IX, Fig. 9).

As a rule the segmenting bodies correspond in size with the larger full-grown organisms, filling somewhat more than one-half of the corpuscle. Not infrequently, however, one may see in the same blood segmenting forms hardly half this size. This suggested the possibility that we may be dealing with two distinct organisms. Celli and Sanfelice think that the larger and smaller segmenting bodies belong to separate varieties; in fact, this is, as has been mentioned, the most important point of distinction between their second and third varieties of parasites, those with cycles of development of respectively mean and rapid duration. Labbé, on the other hand, because he finds the larger and smaller forms always associated, while, at the same time, others of intermediate size occur, comes to the conclusion that they are forms of one organism, a parasite which under certain circumstances may sporulate at a period somewhat earlier than usual in its life history. This explanation is in accord with what we have observed.

From analogy with what we know of the tertian and quartan parasites of human malaria it is natural that one should suspect that these organisms might, in a similar manner, undergo a cyclic development in groups, all the members of each group being in approximately the same phase of development. This, however, is apparently not the case. In a specimen of blood in which the parasites are fairly abundant one finds as a rule nearly all the forms that have been described, though not infrequently one form predominates. For this reason we have been able to arrive at no definite conclusion as to the length of the cycle of development.

In addition to the full-grown organisms, which correspond in size to the largest segmenting bodies, certain other forms of a characteristic appearance are usually to be found. These are as a rule much larger than any of the segmenting bodies and are apparently not destined to segment, at least in the ordinary way. The nucleus of the containing corpuscle is pushed to the extreme periphery; the size of the parasite

is usually such that the corpuscle is distorted. Often one finds the parasite surrounded by a rim of corpuscle from which the nucleus has been extruded. As a rule the corpuscle or what is left of it is decolorized. These bodies are much less refractive than the smaller forms and stand out, therefore, much less distinctly from the corpuscle. The pigment is not collected into a clump, but is distributed throughout the organism as discrete granules. In specimens that have been fixed and stained with eosin and methylene blue, best by the method of Romanowsky, these bodies remain almost completely unstained (Plate IX, Fig. 7); occasionally a stained peripheral rim may be made out surrounding a large unstained central area.

These are, apparently, the bodies that Grassi and Feletti have described as a separate species of organism under the name *Haemamoeba relicta*. They believed them to segment as do the other forms. Celli and Sanfelice, on the other hand, have found them only in connection with their parasites with rapid development and regard them as forms analogous to the crescents of æstivo-autumnal fever.

We have found these bodies associated with the irregular type of organism whenever the parasites were at all abundant. The fact that they are as a rule larger than any of the segmenting bodies, as well as the fact that they stain very faintly, while the segmenting bodies always contain much chromatophilic substance, is in favor of the view that they are sterile forms.

In specimens of blood containing only the irregular form of parasite we have a number of times met with flagellate bodies very closely resembling those seen in human malaria. A round extracorpuscular body, usually lying beside the free nucleus of a red blood corpuscle, is seen to acquire an active vibratory motion. This motion is due to a number of actively motile flagella which have appeared at the periphery. The flagella are similar in appearance to those which occur in the malarial parasites of human beings. They are several times the length of the diameter of the body from which they arise. At their extremities they often show slight olive-shaped swellings. Alterations in the shape of the body of the organism are common; a constriction may apparently divide it into two circular bodies touching at but

one point (Plate IX, Fig. 10), while later it again may take on its original circular shape. In time the flagella disappear and the body becomes motionless. The presence of the free nucleus beside the extracellular body indicates, in view of what is often seen to occur with the elongate parasite (p. 94), that the organism may have escaped from the corpuscle after the preparation of the specimen. The size of these extracellular parasites which develop flagella, and the fact that they are not so refractive as the smaller intracellular bodies, suggest that they are possibly identical with the large palely staining bodies with discretely distributed pigment granules.

The above described parasites we have found in the English sparrow (*Passer domesticus*), swamp sparrow (*Melospiza georgiana*) and red-winged blackbird (*Agelaius phoeniceus*). No differences were made out between the organisms found in these different species of birds.

Elongate Parasite (*Halteridium of Labbé*). The second variety of parasite which we have observed, the elongate organism which lies curved alongside the nucleus, is markedly different from the irregular form which we have just considered. Since we have found that distinct morphological differences were to be made out between the parasites of this variety present in different birds, as a type that variety will be described which was found in the greatest number of hosts. In the English sparrow (*Passer domesticus*), song sparrow (*Melospiza fasciata*) and crow (*Corvus americanus*) were found organisms morphologically indistinguishable. These may be regarded as typical examples of the halter-shaped parasite.

The youngest non-segmented organisms closely resemble corresponding forms of the irregular parasite; pigment soon appears as small brownish granules. The small forms vary in shape, usually they are round or oval. We have not observed amœboid movement, though very short processes are often present. These young forms stain only in their peripheral portions, as do the similar stages of the irregular parasite; the central part, which contains no pigment, remains unstained (Plate IX, Fig. 11). As the parasite grows it becomes elongated. Having reached a certain size it appears to adhere closely

to the nucleus of the corpuscle. When somewhat longer than the nucleus the parasite is of semilunar shape with pointed ends hugging the nucleus (Plate IX, Fig. 12). With further growth the extremities of the parasite become more bulky and rounded and finally fill almost the whole of both ends of the corpuscle. The full-grown body then lies along one side of the normally placed nucleus and curves over its two extremities (Plate IX, Figs. 13-14). The pigment of these large forms appears as very coarse oval highly refractive granules of a yellowish-brown color, distributed irregularly throughout the parasite or collected towards the two ends. The corpuscle containing the very large forms is usually somewhat decolorized, but otherwise looks normal.

Additional information as to the morphology of the organism can be obtained from specimens fixed and stained. The method which we have used most extensively is that of Romanowsky. The material which in the young forms takes up the stain increases in amount with growth, so that in the large bodies it is scattered throughout the parasite. These large parasites take a deep stain which is not, however, homogeneous, but results from the presence of a number of small oblong chromatophilic particles; these chromatophilic granules are well seen in specimens fixed by heat, stained with safranin and partially decolorized in alcohol.

In forms but little longer than the nucleus of the corpuscle there becomes visible an approximately circular, clear-cut area, which stands out conspicuously from the fact that it remains unstained. Within this pale area one or more small bodies may at times be seen which, by the methods employed, take a very faint stain. Various observers have described such a structure as a vesicular nucleus containing nucleoli.

Besides these deeply staining forms, and at times in almost equal abundance, we have found full-grown bodies which remain almost completely unstained (Plate IX, Figs. 14-16). The longest of these are somewhat larger than the deeply staining bodies. The pigment granules are situated in the two extremities; this peculiarity is explained by a further fact which staining brings out. The whole of

the middle portion of the organism is occupied by a very large, clear, almost completely unstained space, sharply marked off from an exterior layer, thickest at the two ends, which takes a faint blue stain. The sharp outline and position of the central clear space indicate that it corresponds to the so-called vesicular nucleus of the other forms. The outer faintly staining layer represents apparently the body of the parasite containing a very small amount of chromatophilic substance. It is in the latter portion that the pigment is situated, and since this portion is thickest at the extremities of the parasite, it is here that the pigment tends to collect.

When the blood is drawn large numbers of these swollen, palely staining forms quickly assume a circular outline and escape from the containing red corpuscle. In stained specimens of these circular bodies (Plate IX, Fig. 16) the central unstained area is surrounded by the external palely staining material which now forms a rim of uniform thickness. In this peripheral rim lies the pigment. In many of these bodies, which have become completely extracellular, the distinction of internal non-staining area and external palely staining material is lost and the pigment is scattered throughout the body.

The question arises whether these non-staining bodies are forms which after maturing have, by a sort of degenerative process, lost their affinity for stains, or are forms which have never acquired any considerable amount of chromatophilic substance. In favor of the latter hypothesis is the fact that smaller forms which take but little color are frequently found in the same specimen with the larger bodies.

Reproduction of the elongate form of intracorpuscular parasite does not, according to our observations, take place in the circulating blood. Neither in the English sparrow, song sparrow nor crow infected with this parasite have we found bodies showing any indication of segmentation. The bone-marrow and internal organs of these birds were not, however, examined in the fresh state.

Labbé is the only observer, so far as we know, who has definitely described the segmenting stage of the halter-shaped organism. In the lark, particularly in the bone-marrow and spleen, he found segmenting bodies. According to him the nucleus of the parasite divides into two,

the daughter nuclei occupying the two extremities of the organism. The two extremities containing the secondary nuclei then divide, each into from six to fifteen segments. We have not observed this process, although in one or two instances in the owl (*Bubo virginianus*) we have seen parasites with two of the so-called nuclei.

In the bone-marrow of a swamp sparrow (*Melospiza georgiana*) infected with elongate organisms, which to be sure differ somewhat from those which we have just described in the English sparrow, song sparrow and crows, we have, however, encountered bodies in process of segmentation. The organisms, as seen in preparations of bone-marrow teased in normal salt solution, had assumed a round form with the pigment collected into a central block, and had divided into a number of round segments arranged about the pigment mass.

From analogy with the segmenting bodies of the irregular parasite which stain well, one would suppose that it is the deeply staining bodies of the elongate parasite that segment, while for the same reason it seems improbable that the palely staining forms which are characterized by swollen vesicular body undergo segmentation.

The process of *flagellation* presented by the elongate organism is a most remarkable phenomenon. In birds in which these organisms are abundant the whole process may be followed with the greatest facility; in almost every field of the microscope (Leitz, Obj. oil im. 1/12; Oc. 3) one or more flagellate bodies may be observed. To see the whole process the specimen of blood must be prepared and brought under the microscope with considerable rapidity. Certain of the full-grown bodies which lie curved along one side of the nucleus of the corpuscle are soon seen to collect themselves into an oval, then into a circular form, causing a marked bulging protrusion from that side of the containing corpuscle. As one watches the corpuscle its body disintegrates; the hæmoglobin seems to fade away under the eye and a round organism is left lying beside the nucleus of what was shortly before the containing corpuscle. Very soon the round extracellular body acquires an active vibratory motion as the result of the presence of two, three, four, or even more, very active flagella, which suddenly make their appearance at the periphery (Plate IX, Fig. 17). The

pigment granules take on a very lively dancing motion. Changes in the shape of the body of the organism often occur. As with the flagellate forms of the irregular variety, a constriction may divide the parasite into two circular bodies connected at but one point. After a while individual flagella not infrequently become detached and float away, continuing their active serpentine movements. After persisting for some time the flagella become less active and finally disappear, by exactly what process cannot be said in every case. The pigment loses its motion.

The liberation of the intracorpuscular parasites appears to be the direct consequence of the changes which take place in the blood as a result of abnormal conditions produced in making the specimens, such as, for example, the rapid reduction of the temperature of the blood, the chemical changes in the plasma accompanying coagulation and the changes resulting from exposure of the blood to the air. It cannot be definitely determined whether the liberation of the parasite is primarily the result of disintegration of the containing corpuscle whose resistance to external changes has been weakened by its presence, or whether it may be due to the action of the parasite which, meeting with the new conditions, contracts into a globular form, and in so doing breaks from the corpuscle. It may be said that in specimens of birds' blood, however carefully made, a certain number of corpuscles which do not contain parasites disintegrate; the hæmoglobin is dissolved in the plasma and the nucleus alone remains. Where little care is used in the preparation of the specimen large numbers of these free nuclei are seen. Where organisms are contained in one of these disintegrating corpuscles they apparently become free, assuming a round form even though they have not reached the size of those which ordinarily escape. In such a case certainly the liberation of the organism is not primarily the result of its own activity, but of the distintegration of the corpuscle. A similar explanation probably holds for the larger forms.

A certain number of the organisms that become free, but by no means all, develop flagella. We have, it will be remembered, described two forms of full-grown organisms: the deeply staining bodies with small nucleus-like areas and the palely staining forms with the

large vesicular structure, which are constantly present in the blood of birds infected with the elongate type. Do both of these forms develop flagella? Since this question has suggested itself to us we have not been able to obtain a bird with sufficient number of forms capable of developing flagella to enable us to arrive at any conclusion.

If blood be allowed to stand some time before the dried preparation is made, it will be found that both the pale and the deeply staining parasites become free. This is, however, of no especial significance; under such conditions many non-parasitiferous red corpuscles also disintegrate. As far as we have been able to judge, the palely staining forms are the first to become free. Sakharoff (26), who with Danilewsky thinks that the flagellate forms are a separate species of organism, states that the organisms which flagellate are distinguished from those that do not by the possession of a very large nucleus. These bodies with large nuclei would appear to be identical with our swollen palely staining forms, which we have been rather inclined to regard as the variety which develop flagella.

Varieties of the Elongate Parasite. There is considerable diversity of opinion among observers as to the existence of distinct species of the organisms under consideration. Celli and Sanfelice observed certain special differences in the size and shape of the elongated parasites. For example, in some birds the extremities of the full-grown forms were more blunt than in others; in the barn owl (*Strix flammea*), and in the brown owl (*Syrnium aluco*), the organisms contained numerous conspicuous refractive particles scattered between the pigment granule.

Labbé, in his *Halteridia* of the lark, chaffinch, starling and jay, found differences in shape and refraction in different birds. These authors do not, however, emphasize morphological differences as indicating the existence of separate species of organisms. Celli and Sanfelice, however, think their failure to transfer organisms by inoculation from one species of bird to another, indicates that there are distinct species of parasites peculiar to the different hosts.

Labbé finds but one species of Halteridium which, in view of unsuccessful inoculation from one species of bird to another, he thinks is

composed of numerous varieties peculiar to the special hosts. Little reliance can, however, be placed upon unsuccessful inoculations from one species of bird to another as pointing to specific differences, since more recently Di Mattei, as already mentioned, has in a large number of carefully conducted experiments wholly failed to transfer infection from bird to bird of the same species.

The elongate parasites which we have encountered have presented in different birds distinct structural peculiarities which in certain cases are very striking. In several instances, however, we have observed similar differences in the organisms of a single bird. Hence, even though the morphological differences between the parasites are greater than those existing between the tertian and quartan organisms of human malaria, the lack of extended observations makes it impossible to establish distinct species. Nevertheless, we will call attention to the chief varieties which we have noted.

(1) In an English sparrow and a song sparrow obtained near Baltimore, and in three crows from near Toronto, Canada, were found organisms in every respect identical (Plate IX, Figs. 11-17). These, selected as an example of the halter-shaped organism, have already been described. They present certain distinctive features. When the young forms have reached a length but little greater than that of the nucleus of the corpuscle, unlike the other varieties, they adhere closely to the nucleus. The extremities of the organism, at first pointed, grow over those of the nucleus, hugging them closely. At this time the body is crescentic; later its two ends become rounded and bulky. The most striking feature, however, of this variety is the pigment. This is scattered through the organism as very coarse, yellowish brown, highly refractive oval granules; these in the full-grown forms are several times the size of those usually found. The large palely staining forms with swollen vesicular structure are found in great abundance. Flagellate bodies also are seen here in great numbers.

(2) In a great horned owl (*Bubo virginianus*) an organism (Plate IX, Figs. 18-22) was observed which differed markedly from that above described. In common with the other varieties it begins its growth as a small non-pigmented body, situated as a rule in one or

other end of the red corpuscle. With increase in size it often comes to occupy a large part of the extremity and may be so large as to slightly displace the nucleus, never, however, pushing it into the opposite end. At the same time growth proceeds longitudinally along one side of the nucleus toward the other end of the corpuscle. When full-grown the organism occupies every available portion of the corpuscle, displacing the nucleus laterally. The pigment consists of numerous small spherical granules which are almost black.

In fresh specimens in the half-grown and full-grown forms there may be seen between the pigment granules numerous, highly refractive, spherical particles. In specimens stained with methylene-blue and eosin these bodies appear as completely unstained round areas within the blue ground of the organism. The vesicular body described as a nucleus stands out clearly in stained specimens; within, one or more palely staining particles may be made out. Palely staining parasites are abundant. Flagellate forms with from three to five flagella are numerous. Irregularity in the shape of the flagellate body is common; bud-like processes often appear at the periphery and may give origin to flagella.

(3) In a swamp sparrow (*Melospiza georgiana*) was found an organism (Plate IX, Figs. 23 and 24) markedly unlike those already described. When full-grown it appears as a rather slender body of almost uniform width lying along the periphery of the corpuscle, at one side of the nucleus but not touching it. The border of the parasite toward the nucleus has an irregular appearance. Pigment is present in fine round granules. Conspicuous refractive bodies are not present. Although intracorpuscular organisms were seen to assume a circular shape and become free, flagellation was not observed, possibly on account of the small number of parasites present. In the bone-marrow of this bird, as already mentioned, segmenting bodies were found.

(4) In several red-wing blackbirds (*Agelaius phoeniceus*) there were seen organisms (Plate IX, Figs. 25 and 26) resembling somewhat those of the last mentioned form. They differ from these, however, in not being so slender and in their lack of uniformity in width. As

a rule they do not lie along the periphery of the corpuscle, but are often close to the nucleus. Those forms which have not completed their growth show as a rule a somewhat club-like shape, one end being more bulky than the other; growth apparently takes place along the side of the nucleus from the larger end as a starting point. In the largest forms both ends are clubbed. The pigment is in small spherical granules not infrequently collected into one or both extremities.

(5) In one red-wing blackbird (*Agelaius phoeniceus*) occurred an organism (Plate IX, Figs. 27-29) which differed from that last mentioned. The extremities of the larger forms are not clubbed, but are pointed, curling about the ends of the nucleus of the corpuscle, and with further growth approach one another on the side of the nucleus opposite to the main bulk of the parasite. In certain cases the two extremities meet one another and fuse, thus forming a complete ring surrounding the nucleus. Pigment is present as round granules, usually scattered throughout the organism, at times collected in a more or less definite clump. Associated with this variety of organism is a form (Plate IX, Fig. 29) very similar to bodies which Kruse (15) has described and pictured, regarding them as, in all probability, segmenting forms. They are seen in specimens stained by the method of Romanowsky as elongated bodies lying along one side of the nuclei; the pigment is collected into a fairly compact mass which occupies the middle portion. The body of the parasite is composed of a number of round areas which take no stain, surrounded by stained protoplasm. The whole has the appearance of being composed of a number of bodies with central, non-staining area and a peripheral staining rim. Whether or not these are really segmenting bodies cannot be definitely stated.

The above mentioned differences among the organisms observed certainly suggest the possibility of the existence of distinct species. On the other hand, it may be that a single organism is modified by peculiarities in the conditions which it meets in different species of host or in the same host under varying conditions. But even if the above described peculiarities do not indicate specific differences among the organisms concerned, they are nevertheless worthy of note as evidence of a very remarkable polymorphism.

DESCRIPTION OF PLATE IX.

The figures, those of flagellate forms excepted, are drawn from specimens stained with methylene blue and eosin by the method of Romanowsky (modified by Heppener). The figures of flagellate forms are drawn from fresh specimens of blood. Leitz, Oc. 3; Obj., oil im. $\frac{1}{12}$.

Figs. 1-10.—Irregular parasites found in the English sparrow, swamp sparrow and red-winged blackbird.

Figs. 11-17.—Elongate parasites, variety (1), found in the English sparrow, song sparrow and crow.

Figs. 18-22.—Elongate parasites, variety (2), found in the great horned owl.

Figs. 23 and 24.—Elongate parasites, variety (3), found in the swamp sparrow.

Figs. 25 and 26.—Elongate parasites, variety (4), found in the red-winged blackbird.

Figs. 27-29.—Elongate parasites, variety (5), found in the red-winged blackbird.

For fuller description of these figures see the text.

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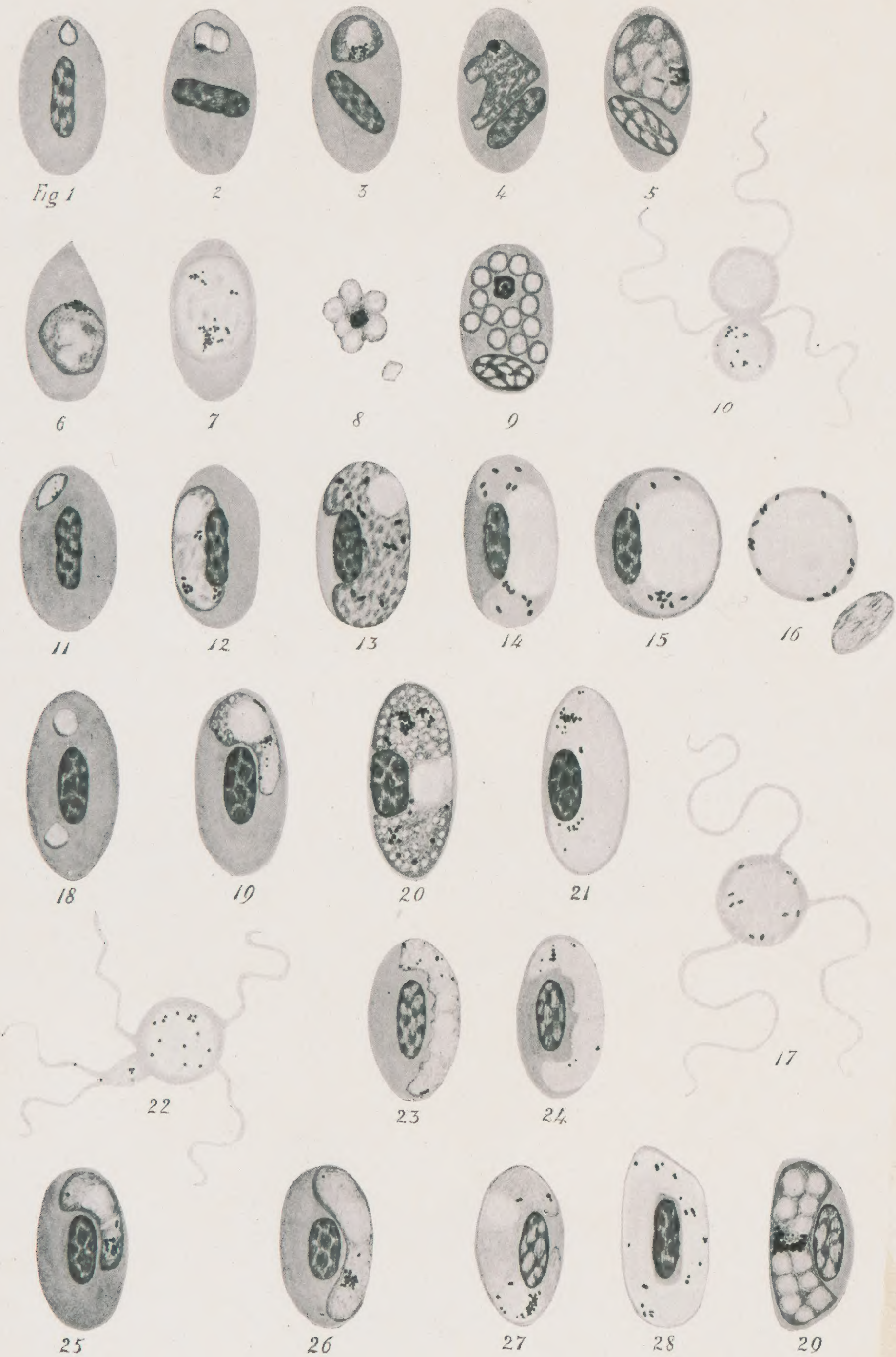
Fig 1
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